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Toxicity of the Herbicide Glufosinate-Ammonium to *Tetranychus urticae* (Acari: Tetranychidae) under Laboratory and Field Conditions

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Abstract: The toxicity of herbicides widely used in apple orchards to the two-spotted spider mite (*Tetranychus urticae*) was evaluated in laboratory and field studies. In a laboratory study with susceptible *T. urticae*, glufosinate-ammonium was highly effective against larvae, protonymphs and adults, but non-toxic to eggs. Its efficacy was much greater than that of the commonly used acaricide azocyclotin. The immatures died within 24 h after treatment, suggesting that the nymphicidal action may be attributable to a direct effect rather than an inhibitory action of chitin synthesis. Glufosinate-ammonium showed a positive temperature coefficient of toxicity against *T. urticae* adults at six temperatures from 10 to 32°C, being more toxic at higher temperatures. Very low levels of resistance to the herbicide were observed in the seven field-collected *T. urticae* populations resistant to various acaricides. Treatment with glufosinate-ammonium did not cause a repellent response from either adults or immature stages of *T. urticae*. Paraquat dichloride and glyphosate were ineffective against all stages of *T. urticae*. In a field study of a population of *T. urticae*, glufosinate-ammonium when sprayed to weeds caused significant decrease in *T. urticae* population densities in apple trees for nine weeks after treatment, as compared with the control. Thereafter, a single application of standard acaricides to apple foliage greatly reduced population densities, although there was no difference in the densities between the glufosinate-ammonium-treated and control plots. Based upon laboratory and field data, two single treatments with glufosinate-ammonium to weeds in May and a selective acaricide to apple trees in July may be used to prevent damage by *T. urticae*.

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1 INTRODUCTION

Apples (*Malus pumila dulcissima* Koidz) are one of the major fruit crops in Korea where their cultivation area was approximately 52 000 ha in 1994, and is increasing every year due to their economic significance.[1] Among six species of spider mite pests of apple in Korea, the most important is the two-spotted spider mite (*Tetranychus urticae* Koch).[2,3] If not managed properly from the early growth-stage of this fruit crop, this mite species adversely affects quantity and quality such as weight, sugar content, hardness and acidity of fruits when adults and immature stages feed excessively on leaves.[4]

Current control of *T. urticae* populations in Korea is primarily dependent upon repeated applications of acaricides or acaricide groups. Between 1990 and 1994, an average of nearly 0·23 million kg per year of acaricides was used in Korea for controlling this spider mite species.[5] Although they have effectively controlled this spider mite, their continued use on apple orchards for several decades has disrupted biological control by natural enemies and led to resurgences in spider mite populations[6] and the development of widespread resistance to various types of acaricide.[7–9] Many of the 36 acaricides registered for use on apple[5] have failed to provide adequate control of this spider mite after two or three years of use in the field. Besides these problems, factors such as higher labour costs, pesticide application costs, safety, and adverse effects on environment make apples difficult to cultivate. This economic consideration, and the decreasing efficacy and increasing concern over adverse environmental effects of the earlier types of acaricide have brought about the need for the development of new types of selective control alternatives or alternative control methods with reduced use of synthetic pesticides.

In the laboratory and field studies described here, we have assessed the effectiveness against *T. urticae* of three widely used herbicides (glufosinate-ammonium, paraquat dichloride and glyphosate) and three acaricides, to avoid unnecessary pesticide applications. The effect of temperature on the acaricidal activity of glufosinate-ammonium was also investigated in relation to its practical use in apple orchards.

2 MATERIALS AND METHODS

2.1 Chemicals

Herbicides and acaricides used in this study were as follows: glufosinate-ammonium 180 g litre^{-1} SL; paraquat dichloride 245 g litre^{-1} SL; glyphosate-isopropylammonium 410 g litre^{-1} SL; fenpyroximate 30/propargite 100 g litre^{-1} EW; tebufenpyrad 25/diafenthiuron 200 g kg^{-1} WP; and azocyclotin 250 g kg^{-1} WP. These chemicals were supplied by Kyungnong Corporation, Seoul, Korea.

2.2 Laboratory studies

A total of seven field-collected populations of *Tetranychus urticae* Koch were used in this study. The collection sites and the susceptibilities of these mites to various acaricides were as previously described.[7,9] A susceptible strain, obtained in 1993 from Dr N. Motoyama of Chiba University, Chibaken, Japan, was used as the standard for comparison. The mites were reared on kidney bean (*Phaseolus vulgaris humilis* Alefeld) seedlings (three weeks after germination) in stainless steel trays (33 × 37 × 5 cm), and maintained at 25(±1)°C and 40–60% relative humidity (RH) under a 16 : 8 h light : dark photoperiod.

To synchronize the developmental stages, adults were placed on kidney bean plants with a fine brush and allowed to lay eggs for 3 h, after which time the adults were removed with an aspirator. The infested plants were held under conditions as above. The stages tested consisted of eggs, immature stages (larvae and protonymphs), and adults. The numbers of each stage on each leaf were counted before treatment.

Effects of the test chemicals on each stage of the susceptible *T. urticae* were determined by spraying a single leaf of infested kidney bean plant, and each treatment was replicated three times. The other leaves were removed from the plant before treatment. Sprays were applied with a glass spray unit connected to a forced air supply (Pacific Chemical, Seoul, Korea). The chemicals were tested at the rates of 4·740, 2·370, 1·185 and 0·593 mg AI per single leaf attached to each plant. Treated plants were maintained under the above conditions for 24 h. The leaf in each treatment was then placed onto a single leaf of fresh kidney bean plant because the herbicides tested caused leaf wilting.

Susceptibility of adults of the seven field-collected *T. urticae* populations to glufosinate-ammonium was determined as above.[7,9] A resistance ratio (RR) was calculated according to the formula RR = LC_{50} of the field-collected population/LC_{50} of the susceptible strain. RR values of <10, 10–40, 41–160 and >160 were considered as low, moderate, high and very high resistance, respectively.

The activity of glufosinate-ammonium against the susceptible *T. urticae* adults was examined at six temperatures from 10 to 32°C using the leaf-mite dipping method. Each treatment was replicated three times. Leaves of kidney bean (three weeks after germination) grown in the greenhouse were collected and disks (3 cm dia.) were punched from each leaf. Three leaf disks with adults were dipped in a test suspension for 10 s. Treated disks were maintained in incubators (VS-1203PL, Vision Scientific, Suwon) at 10, 13, 18, 22, 25

and 32°C, with RH and photoperiod as before for 24 h. The disk in each treatment was then placed onto a fresh leaf disk because of leaf wilting caused by glufosinate-ammonium.

The toxicity of the test chemicals on the eggs was based on the number of unhatched eggs six days after treatment (DAT). Larval mortalities were assessed on the basis of failure to complete the next ecdysis. Evaluation of adulticidal activity was made 2 DAT. Mites were considered dead if appendages did not move when prodded with a fine brush. Data from all bioassays were corrected for control mortality using Abbott's formula.[10] LC_{50} values and slopes were calculated by probit analysis.[11]

2.3 Field studies

Glufosinate-ammonium and the acaricides were field tested in a 17-year-old commercial apple orchard in Whasung-Gun (Kyunggi Province), planted with 'Fuji' trees in rows 6 m and 3 m apart. The trees ranged in height from 2·5 to 3 m. In all experiments, standard orchard fungicide programs were maintained throughout the growing season. The treatment was applied as foliar sprays to five-tree replicates. All treatments were applied with an airblast sprayer (JPS-64-A, Chungang Industrial, Seoul, Korea) equipped with one nozzle, calibrated to deliver 2200 litre ha^{-1} of liquid. The different chemicals were applied at doses based on company-recommended field application rates: glufosinate-ammonium, 540; fenpyroximate + propargite, 87; tebufenpyrad + diafenthiuron, 225; azocyclotin, 162·5 mg AI $litre^{-1}$.

Glufosinate-ammonium application on weeds was initiated on 17 May 1995 when *T. urticae* adults were observed on weeds in all field plots, but not observed on apple foliage of all plots. The dominant weed species were as previously described.[12] Applications of the three acaricides to apple foliage were made on 25 July 1995. Trees were sprayed when *T. urticae* population densities reached three to four mites per leaf in the glufosinate-ammonium-treated plots. Population densities of *T. urticae* were estimated at one-week intervals after treatment by sampling 30 leaves from each of five trees per plot from the periphery of the canopy at a height of 1·5–2·0 m. Mobiles were counted using a binocular dissecting microscope. Controls received water.

In a separate experiment, fenpyroximate + propargite (174 mg AI $litre^{-1}$), tebufenpyrad + diafenthiuron (450 mg AI $litre^{-1}$) and azocyclotin (325 mg AI $litre^{-1}$) were applied to the young leaves of 17-year-old 'Fuji' apple trees on 12 May and to the young fruits on 29 May 1995. The presence or absence of leaf burn and damage in treated trees was assessed at five-day intervals for 30 days following each acaricide application and compared with the untreated trees.

2.4 Statistical analysis

The percentage of mortality was determined and transformed to arcsine values for analysis of variance (ANOVA). The population densities were subjected to ANOVA. Treatment means were compared and separated by Scheffe's test at $P = 0{\cdot}05$.[13]

3 RESULTS

3.1 Laboratory studies

The toxicity of the test herbicides to eggs, immature stages (larvae and protonymphs), and adults of the susceptible *T. urticae* was compared with that of azocyclotin (Table 1). Significant differences between toxicities of the test chemicals were observed. Glufosinate-ammonium was highly effective, even at the lower concentrations, against immature stages and adults, but ineffective against eggs. Its efficacy was much greater than that of azocyclotin. The immatures died within 24 h. Additionally, treatment with glufosinate-ammonium did not cause a repellent response from either adults or immature stages of *T. urticae*. Paraquat dichloride and glyphosate were non-toxic to all stages of *T. urticae*. Unlike glufosinate-ammonium, azocyclotin was effective on all stages of this species at a concentration of 4·740 mg AI $litre^{-1}$.

Susceptibility of seven field-collected populations of *T. urticae* to glufosinate-ammonium is shown in Table 2. The susceptibility of these field populations to various acaricide types has already been reported.[7,9] RRs for these populations ranged from 2·3- to 10·5-fold indicating low levels of resistance to this herbicide.

Table 3 shows the toxicity of glufosinate-ammonium at each temperature examined. The compound showed a positive temperature coefficient of toxicity against the susceptible adults at six temperatures from 10 to 32°C, being more toxic at higher temperatures. Its acaricidal activities at 25 and 32°C were respectively 17 and 20 times that at 10°C.

3.2 Field studies

The effect of glufosinate-ammonium on the *T. urticae* population densities in apple trees when sprayed on weeds on 17 May is shown in Table 4. Significant differences in the population densities between the treated and control plots were observed for each period investigated. The mean population densities of glufosinate-ammonium treatments were not significantly different ($P = 0{\cdot}05$) until nine weeks (18 July) after treatment. *T. urticae* was not observed in plots treated with glufosinate-ammonium during the first seven weeks (4 July), whereas control plots were highly infested with this mite species. As compared with control plots, the

TABLE 1
Toxicity of Test Chemicals to Susceptible *Tetranychus urticae:* Foliar-Spray Bioassay on Single Leaf Attached to Kidney Bean Plant

Chemical	*Rate (mg AI per leaf)*	*Mortality (%)[a] (±SE)*							
		n	*Egg*	*n*	*Larva*	*n*	*Protonymph*	*n*	*Adult*
Glufosinate -ammonium	0·593	400	0d	125	65·8 (±7·2)d	138	52·6 (±5·2)d	89	66·0 (±3·6)de
	1·185	400	0d	398	100a	216	97·7 (±0·5)b	151	82·0 (±0·8)cd
	2·370	336	0d	207	100a	161	100a	183	94·0 (±4·3)b
	4·740	355	0d	242	100a	147	100a	143	100a
Paraquat dichloride	0·593	321	0d	203	0e	155	0e	108	14·7 (±2·6)f
	1·185	359	0d	197	0e	167	0e	94	0g
	2·370	400	0d	225	0e	244	0e	147	0g
	4·740	400	0d	198	0e	179	0e	183	0g
Glyphosate	0·593	355	0d	167	0e	161	0e	179	0g
	1·185	385	0d	191	0e	179	0e	91	0g
	2·370	420	0d	268	0e	191	0e	136	0g
	4·740	390	0d	166	0e	206	0e	186	0g
Azocyclotin	0·593	174	17·7 (±4·6)c	234	66·7 (±4·0)c	145	45·7 (±5·9)d	105	49·7 (±7·0)e
	1·185	152	30·3 (±0·2)c	179	80·0 (±3·7)b	190	59·0 (±5·1)d	119	78·0 (±0·8)cd
	2·370	146	56·0 (±0·2)b	293	92·0 (±2·2)a	166	73·7 (±3·4)c	116	91·0 (±2·9)bc
	4·740	332	100a	179	100a	161	100a	134	100a

[a] Means within a column followed by the same letter are not significantly different ($P = 0{\cdot}05$, Scheffe's test).[13] Mortalities were transformed to arcsine square root before ANOVA. Means (±SE) of untransformed data are reported.

TABLE 2
Toxicity of Glufosinate-Ammonium to Susceptible (S) and Field-Collected *Tetranychus urticae* Adults

Population	*Slope (±SE)*	*LC_{50} (95% CL) (mg litre^{-1})*	x^2	*DF*	*RR[a]*
Suwon	1·63 (±0·18)	57·26 (45·26–71·79)	0·92	1	2·3
Kunwi	1·87 (±0·19)	74·35 (60·61–91·52)	1·08	1	3·0
Yeasan	1·37 (±0·17)	103·65 (78·13–134·46)	13·57	1	4·2
Andong	1·71 (±0·34)	118·75 (70·07–156·16)	0·04	1	4·8
Kapyung	2·15 (±0·22)	178·24 (146·87–216·85)	0·45	1	7·3
Oksan	2·20 (±0·25)	194·21 (164·69–227·44)	3·92	2	7·9
Kimcheon	2·92 (±0·36)	258·44 (225·14–294·52)	1·88	1	10·5
S	2·14 (±0·20)	24·60 (20·11–28·96)	5·36	1	—

[a] Resistance ratio, LC_{50} of field-collected population/LC_{50} of susceptible strain.

TABLE 3
Temperature–Toxicity Relationships of Glufosinate-Ammonium against Susceptible *Tetranychus urticae* Adults

Temp. (°C)	*Slope (±SE)*	*LC_{50} (95% CL) (mg litre^{-1})*	x^2	*DF*	*TF[a]*
10	2·56 (±0·61)	424·08 (248·44–571·18)	0·88	2	1·0
13	2·11 (±0·43)	380·81 (241·30–504·54)	1·83	2	1·1
18	2·29 (±0·41)	82·78 (55·37–106·49)	1·53	1	5·1
22	1·62 (±0·25)	41·43 (29·86–53·95)	0·16	3	10·2
25	2·21 (±0·24)	24·94 (20·48–29·21)	1·34	2	17·0
32	2·98 (±0·37)	21·29 (17·96–24·31)	0·02	1	20·0

[a] Toxicity factor relative to 10°C.

TABLE 4
Effect of Glufosinate-Ammonium on Field Population Densities of *Tetranychus urticae*

Treatment[a]	*Rate* (*mg AI litre*$^{-1}$)	*Population density* ($\pm SE$)[b]				
		July			*August*	
		4	*11*	*18*	*1*	*15*
Glufosinate-ammonium only	540	0b	21·9 (±2·9)b	43·3 (±3·0)b	424·4 (±12·3)a	581·9 (±32·0)b
Fenpyroximate + propargite	87	0b	19·1 (±1·2)b	64·1 (±2·7)b	9·6 (±2·3)c	0c
Tebufenpyrad + diafenthiuron	225	0b	18·2 (±2·2)b	66·5 (±4·1)b	92·6 (±7·2)b	4·3 (±1·2)c
Azocyclotin	162·5	0b	25·3 (±2·4)b	76·0 (±2·6)b	81·6 (±5·2)b	2·7 (±0·3)c
Control		111·3 (±8·5)a	222·1 (±6·1)a	393·1 (±19·2)a	454·6 (±7·1)a	662·8 (±9·2)a

[a] Applications of glufosinate-ammonium to weeds and the test acaricides to apple foliage were done on 17 May and 25 July 1995, respectively.
[b] Means within a column followed by the same letter are not significantly different ($P = 0{\cdot}05$, Scheffe's test).[13]

glufosinate-ammonium treatments significantly reduced population densities by 89–92 and 81–89% eight (11 July) and nine weeks (18 July) after treatment, respectively. On 23 July, the mite population densities of the treated plots had increased (3–4 mites per leaf). Accordingly, foliar applications of the test acaricides were made on 25 July. By one week after acaricide treatment (11 weeks after glufosinate-ammonium treatment), the population densities receiving applications of fenpyroximate + propargite, tebufenpyrad + diafenthiuron and azocyclotin were reduced by 98, 80 and 82%, respectively, whereas there was no difference in the densities between the glufosinate-ammonium-treated and control plots. Differences were also detected ($P = 0{\cdot}05$) among treatments by two weeks after acaricide treatment (13 weeks after glufosinate-ammonium treatment).

No symptoms of phytotoxicity were observed on 17-year-old 'Fuji' apple trees that were exposed to two applications of any of the three acaricide treatments.

4 DISCUSSION

In the laboratory study with all stages of *T. urticae*, glufosinate-ammonium exhibited excellent acaricidal activity against immature stages and adults, but showed no toxicity to eggs. The immatures died within 24 h, suggesting that the nymphicidal action might be attributable to a direct effect rather than an inhibitory action of chitin synthesis. Low levels of resistance to glufosinate-ammonium were observed in the seven field-collected *T. urticae* populations which had shown low to high levels of resistance to various types of acaricide.[7,9] These results indicate that glufosinate-ammonium may be highly effective against field populations of *T. urticae* and that the acaricidal mode of action of the herbicide may be different from that of the acaricides, although its exact mode of action remains unknown.

Temperature is one of the most important factors which influence toxicity to the target pest. Studies on the effect of temperature on pesticidal activity not only are of practical importance for pest control, but also contribute to the elucidation of pesticide mode of action. A negative temperature coefficient of the activity of DDT and pyrethroids has been reported in various insect species,[14–16] whereas organophosphates have shown a positive temperature coefficient.[17] Schmidt and Robertson[18] also reported that the effect of temperature depends on the test method in the horn fly; permethrin has a negative temperature coefficient when applied topically and a positive one when applied to a treated cloth surface. In our study, glufosinate-ammonium displayed a positive temperature coefficient for mortality when applied by the leaf-mite dipping method, although the possible mechanisms involved remain unknown.

In Korea, *T. urticae* adults move to overwintering sites such as weeds in mid-September to November, and the overwintered adults become active in April.[6] *T. urticae* population densities are very low before June and increase abruptly after July, reaching a peak in August, and thereafter are maintained with lower densities.[6] In our field study, glufosinate-ammonium when sprayed onto weeds caused a significant decrease in *T. urticae* population densities in apple trees for nine weeks after treatment. Thereafter, a single foliar application of the acaricides described (fenpyroximate + propargite, tebufenpyrad + diafenthiuron or azocyclotin) greatly reduced the population densities. Based upon our data and earlier findings, two single treatments with glufosinate-ammonium to weeds in May (average temperature 16°C [5–28°C]) followed by the acaricides described or a selective acaricide such as flufenoxuron[19–21] or AC 303630[22,23] to apple foliage in mid-July would be sufficient for simultaneous control of *T. urticae* and weeds, contrasting with repeated applications of acaricides (four to six times per year) and herbicides (twice a year), which are currently used for controlling *T. urticae* populations and weeds in apple orchards in Korea. As shown in the laboratory test (Table 1), glufosinate-ammonium produced excellent activity against immature and adult stages of *T. urticae*. Additionally, this herbicide did not have repellent activity against adults and immature stages of *T. urticae*, although alphacypermethrin exhibits strong repellent activity towards this species.[19] These results indicate that treatment with glufosinate-ammonium would not cause mite movement to unprotected parts or weeds under apple trees as refuges where their feeding would result in further outbreaks. This may illustrate an advantage of the use of glufosinate-ammonium for effective control of *T. urticae* and weeds to produce a relatively high percentage of undamaged apples that conform to the grade requirements.

In conclusion, glufosinate-ammonium may be a key component of integrated pest management for controlling *T. urticae* and weeds in apple orchards because this herbicide revealed potent acaricidal activity at a low application rate, excellent herbicidal activity against various weeds[12,24,25] and little or no toxicity against natural enemies such as predatory mite species, lacewings and ladybeetles under laboratory and field conditions.[26] Additional benefits such as savings in labour costs, pesticide application costs and lowering of the possibility of environmental contamination would be also expected.

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